OTHER SUBSTANCES

Extracted: acetic acid, anisic acid, benzilic acid, benzoic acid, butyric acid, caprylic acid, cinnamic acid, citramalic acid, citric acid, enanthic acid, fumaric acid, galacturonic acid, gallic acid, glutaric acid, glycolic acid, glyoxylic acid, p-hydroxybenzoic acid, isocitric acid, α-ketoglutaric acid, lactic acid, malic acid, mandelic acid, phenylacetic acid, propionic acid, protocatechuic acid, pyruvic acid, sorbic acid, succinic acid, tartaric acid, valeric acid, vanillic acid, ascorbic acid

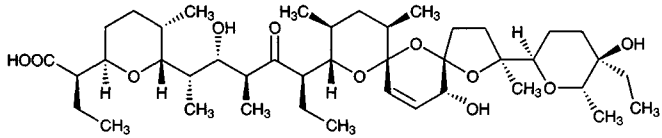
KEY WORDS

derivatization

REFERENCE

Mentasti,E.; Gennaro,M.C.; Sarzanini,C.; Baiocchi,C.; Savigliano,M. Derivatization, identification and separation of carboxylic acids in wines and beverages by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *322*, 177–189.

Salinomycin



Molecular formula: $C_{42}H_{70}O_{11}$
Molecular weight: 751.01
CAS Registry No.: 53003-10-4, 55721-31-8 (sodium salt)
Merck Index: 8488

SAMPLE

Matrix: albumen, eggs, feed, premix, tissue
Sample preparation: Eggs, feed, premix, tissue. 1 g Sample + 15 mL acetone, vortex for 5 min, centrifuge, decant the acetone layer. Re-extract residue (2x), evaporate the combined acetone layers to dryness. Partition the residue between 25 mL aqueous EtOH (water:EtOH 80:20) and 25 mL petroleum ether (50-110°). Evaporate EtOH fraction to dryness, dissolve residue in MeOH, adjust volume to 500 μL. Inject a 50 or 100 μL aliquot. Albumen. 1 g Sample + 15 mL MeOH, extract for 15 min, centrifuge at 1290 × g at -5°. Evaporate to dryness, dissolve residue in MeOH, adjust to a final volume of 500 μL. Inject a 50 or 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Inertsil ODS-2 (Mandel Scientific)
Column: 250 × 3.2 5 μm CSC-Inertsil 150A/ODS-2 (Mandel Scientific)
Mobile phase: MeOH:water:acetic acid 95:5:0.1
Flow rate: 0.5
Injection volume: 50-100
Detector: UV 520 nm following post-column reaction. The column effluent mixed with vanillin reagent pumped at 1 mL/min. The mixture flowed through a Teflon knitted reactor coil (10 m × 0.5 mm i.d., total volume 2 mL) at 95° to the detctor. (Prepare the vanillin reagent as follows. Slowly and carefully add 20 mL concentrated sulphuric acid (95-98%) to 950 mL chilled MeOH, mix well, allow to cool to room temperature. Add 30 g vanillin with constant stirring. Filter solution, degas using vacuum, and store in amber-colored bottle.)

CHROMATOGRAM

Retention time: <10
Limit of detection: 5 ng/g
Limit of quantitation: 10 ng/g

KEY WORDS

post-column reaction

REFERENCE

Akhtar,M.H.; abou el-Sooud,K.; Shehata,M.A.A. Concentrations of salinomycin in eggs and tissues of laying chickens fed medicated feed for 14 days followed by withdrawal for 3 days, *Food Addit.Contam.*, **1996**, *13*, 897–907.

SAMPLE

Matrix: blood

Sample preparation: Extract plasma with isooctane, add the organic layer to a silica SPE cartridge, wash with dichloromethane, wash with dichloromethane:MeOH (?) 98.5:1.5, elute with dichloromethane:MeOH 90:10, evaporate to dryness, reconstitute with dichloromethane, oxidize with pyridinium dichromate, concentrate, inject an aliquot on to column A and elute to waste with mobile phase A, after 1.85 min divert the effluent from column A on to column B (?), after another 1.8 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 75 mm long C18 (Waters); B 250 mm long C18 (Beckman)
Mobile phase: A MeCN:0.01% HCl 90:10; B MeCN:0.01% HCl 96:4
Detector: UV 225

CHROMATOGRAM

Limit of detection: 5 ng/mL

KEY WORDS

derivatization; plasma; column-switching; heart cut; SPE

REFERENCE

Wei,A.T.; Dimenna,G.P.; Karnes,H.T. HPLC analysis of sodium salinomycin in human plasma using derivatization and heart cut column switching, *Pharm.Res.*, **1992**, *9*, S21.–S21..

SAMPLE

Matrix: eggs, tissue

Sample preparation: 5 g Pulverized frozen tissue or 5 g homogenized whole eggs + 2 mL water + 13 mL MeOH, homogenize for 30 s. Sonicate for 10 min and centrifuge at 2000 g for 10 min. Add 4 mL 100 mM NaOH to a 2 mL aliquot of the supernatant, extract with 2 mL and 1 mL hexane:toluene 50:50 (v/v) for 30 s by inversion, centrifuge at 1500 g for 10 min. Evaporate the combined extracts to dryness under a stream of nitrogen at 60°. Dissolve the residue in 200 μL MeCN:water 75:25. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2 (GL Sciences, Japan)
Mobile phase: MeCN:MeOH:THF:water:trifluoroacetic acid 67:10:10:13:0.1
Flow rate: 1
Injection volume: 20
Detector: MS, VG Platform, Megaflow electrospray probe, positive ion mode, source at 125°, cone voltage 25 V, m/z 773

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 0.5-1 ng/g
Limit of quantitation: 2 ng/g

OTHER SUBSTANCES

Extracted: monensin, narasin

KEY WORDS

domestic fowl; muscle; liver

REFERENCE

Blanchflower,W.J.; Kennedy,D.G. Determination of monensin, salinomycin, and narasin in muscle, liver and eggs from domestic fowl using liquid chromatography-electrospray mass spectrometry, *J.Chromatogr.B*, **1996**, *675*, 225–233.

SAMPLE
Matrix: feed
Sample preparation: 20 g Ground Feed + 200 mL hexane:ethyl acetate 90:10, stir at high speed for 2 h, let stand. Remove an aliquot equivalent to 1 g feed and evaporate it to dryness under reduced pressure at 40°, reconstitute with 2 mL MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 60 × 4.6 3 μm C18 (Hewlett-Packard)
Mobile phase: MeOH:5% acetic acid 90:10
Flow rate: 0.5
Injection volume: 20-25
Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed through a 1.5 mL reaction coil (Kratos Model 510) at 95° to the detector. (Reagent was 40 g/L vanillin in MeOH:sulfuric acid 100:2. Keep in an ice bath and prepare fresh daily.)

CHROMATOGRAM
Retention time: 6.7
Limit of detection: 1 ppm

OTHER SUBSTANCES
Extracted: monensin, narasin

KEY WORDS
post-column reaction

REFERENCE
Lapointe,M.R.; Cohen,H. High-speed liquid chromatographic determination of monensin, narasin, and salinomycin in feeds, using post-column derivatization, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 480–484.

SAMPLE
Matrix: feed
Sample preparation: Pulverize feed in a grinder, mix with EtOH, sonicate for 5 min, filter (0.45 μm), dilute filtrate with EtOH if necessary. 5 mL Filtrate + 1 mL 600 μg/mL 2,4-dinitrophenylhydrazine in MeOH + 1 drop concentrated HCl, heat at 50° for about 3 min, cool to room temperature, make up to 10 mL with EtOH, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeOH:1.5% aqueous acetic acid 94:6
Flow rate: 1
Injection volume: 20
Detector: UV 380, UV 419

CHROMATOGRAM
Retention time: 7.8
Limit of detection: 2-5 ng

KEY WORDS
derivatization; maximum sensitivity at 419 nm

REFERENCE
Mathur,A.K. Determination of salinomycin by high-performance liquid chromatography using a precolumn derivatization technique, *J.Chromatogr.A*, **1994**, *664*, 284–288.

SAMPLE
Matrix: feed, premix
Sample preparation: Feed. Shake 5 g feed with 15 mL MeOH for 2 h, filter, evaporate the filtrate to 3 mL and make up to 10 mL with MeOH, inject a 3 μL aliquot. Premix. Shake 0.5

g premix with 15 mL MeOH for 2 h, filter, make up the filtrate to 50 mL with MeOH, inject a 3 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Separon SGX C18 glass column (Tessek Prague)
Mobile phase: MeOH:water:glacial acetic acid 94:5.9:0.1
Flow rate: 0.02
Injection volume: 3
Detector: UV 592 following post-column derivatization. The column effluent mixed with the reagent pumped at 0.015 mL/min and the mixture flowed through a 150 × 1 reactor containing 40-70 μm acid-washed glass beads at 75° to the detector. (The reagent was 500 mM 4-dimethylaminobenzaldehyde in 1.2 M sulfuric acid in MeOH.)

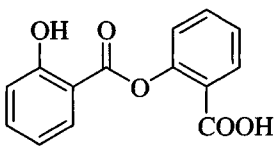
CHROMATOGRAM
Retention time: 16
Limit of detection: 2.2 μg/mL

OTHER SUBSTANCES
Extracted: monensin, narasin

KEY WORDS
derivatization; microbore; post-column reaction

REFERENCE
Fejglova,Z.; Dolezal,J.; Hrdlicka,A.; Frgalova,K. Microbore HPLC determination of polyether antibiotics using postcolumn derivatization with benzaldehyde reagents, *J.Liq.Chromatogr.*, **1994**, *17*, 359–372.

Salsalate



Molecular formula: $C_{14}H_{10}O_5$
Molecular weight: 258.23
CAS Registry No.: 552-94-3
Merck Index: 8491
Lednicer No.: 2 90

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 900 μL 270 mM HCl + 100 μL 100 μg/mL α-phenylcinnamic acid in MeOH + 10 mL dichloromethane, shake at 125 cycles/min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot. Urine. 2 mL Urine + 900 μL 270 mM HCl + 100 μL 100 μg/mL α-phenylcinnamic acid in MeOH + 10 mL hexane, shake at 125 cycles/min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:1% acetic acid 60:40
Flow rate: 2
Injection volume: 25
Detector: UV 300

CHROMATOGRAM
Retention time: 5.8
Internal standard: α-phenylcinnamic acid (8.0)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: aspirin (UV 280), salicylic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Harrison,L.I.; Funk,M.L.; Ober,R.E. High-pressure liquid chromatographic determination of salicylsalicylic acid, aspirin, and salicylic acid in human plasma and urine, *J.Pharm.Sci.*, **1980**, *69*, 1268–1271.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 20 mg/mL solution of bulk aspirin in dichloromethane, inject a 10 μL aliquot as soon as dissolution is complete. Tablets. Prepare a 20 mg/mL solution of ground aspirin tablets in dichloromethane, filter (0.45 μm) immediately, immediately inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 6 μm Zorbax SIL
Mobile phase: Hexane:chloroform:acetic acid 80:19:3 (Before first use pump 10 column volumes of dichloromethane:acetic acid:2,3-dimethoxypropane 96:2:2 through column at 3 mL/min.)
Flow rate: 3
Injection volume: 10
Detector: UV 254

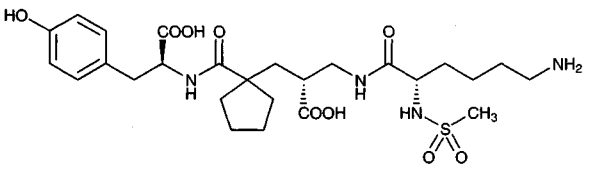
CHROMATOGRAM
Retention time: 2.2
Limit of detection: 5 ppm

OTHER SUBSTANCES
Simultaneous: aspirin, salicylic acid

KEY WORDS
normal phase; tablets

REFERENCE
Pfeiffer,C.D.; Pankey,J.W. Determination of related compounds in aspirin by liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 511–514.

Sampatrilat



Molecular formula: $C_{26}H_{40}N_4O_9S$
Molecular weight: 584.69
CAS Registry No.: 129981-36-8

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut Certify II SPE cartridge with 1 mL MeOH and 1 mL pH 7 phosphate buffer and a Bond-Elut C18 SPE cartridge with 1 mL MeOH and 1 mL water. Add 20 μL 1 μg/mL IS in water and 1 mL pH 7 phosphate buffer to 1 mL plasma, vortex. Add the mixture to the Bond-Elut Certify II cartridge. Wash with 1 mL pH 7 phosphate buffer, 1 mL MeOH:water:trifluoroacetic acid 15:85:0.1, dry under vacuum, elute with 1 mL MeOH: water:trifluoroacetic acid 80:20:0.1. Centrifuge briefly and evaporate to dryness under a stream of nitrogen at 37°. Reconstitute the residue with 1 mL 12% boron trifluoride in MeOH, incubate at 65° for 30 min. Add 2 mL water, evaporate to approximately 1 mL under a stream of nitrogen at 65°, add to the Bond-Elut C18 cartridge. Rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with three 1 mL portions of water, elute into MTBE-rinsed polypro-

pylene tapered tubes with two 1 mL portions of MeOH. Centrifuge briefly, evaporate to dryness under a stream of nitrogen at 37°. Reconstitute in HPLC injection solution, vortex, centrifuge at 10000 rpm for 5 min. Inject an 80 μL aliquot. (HPLC injection solution was MeOH:water 50:50 containing 20 mM ammonium acetate and 5 mM triethylamine.)

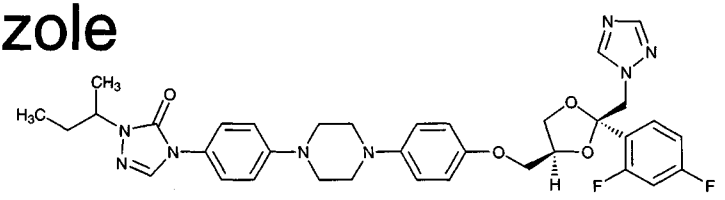
HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S5C6 (A), 30 × 4.6 3 μm (3 × 3CR C18) Perkin-Elmer (B)
Mobile phase: MeCN:20 mM pH 2.25 sodium phosphate buffer containing 2.5 mM octanesulfonic acid 35:65 (A); MeOH:water 80:20 containing 20 mM ammonium acetate and 5 mM triethylamine (B)
Flow rate: 1
Injection volume: 80
Detector: E, ESA Coulochem, analytical electrode 700 mV (A); MS, Perkin-Elmer Sciex API III-plus triple quadrupole, heated nebulizer, positive ion APCI mode, nebulizer 500°, collision gas argon, dwell time 100 ms, Q1 at m/z 613.1 and m/z 597.1, Q3 at m/z 211.4 (B)

CHROMATOGRAM
Internal standard: UK-79.942 (N-(1-(2-carboxy-3-(N2-acetyllysylamino)propyl)-1-cyclopentylcarbonyl)tyrosine, Pfizer Central Research, UK)
Limit of detection: 100 pg
Limit of quantitation: 500 pg/mL

KEY WORDS
plasma; derivatization; SPE

REFERENCE
Venn,R.F.; Kaye,B.; Macrae,P.V.; Saunders,K.C. Clinical analysis of sampatrilat, a combined renal endopeptidase and angiotensin-converting enzyme inhibitor. I: Assay in plasma of human volunteers by atmospheric-pressure ionisation mass-spectrometry following derivatisation with BF3-methanol, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 875–881.

Saperconazole



Molecular formula: $C_{35}H_{38}F_2N_8O_4$
Molecular weight: 672.74
CAS Registry No.: 110588-57-3
Merck Index: 8510

SAMPLE
Matrix: blood
Sample preparation: 250 μL serum + 50 μL 0.3 N barium hydroxide + 50 μL 0.4 N zinc sulfate + 1 mL MeCN, vortex, centrifuge at 3521 g for 15 min, evaporate the supernatant to dryness under a stream of nitrogen, reconstitute with 250 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 7.5 × 4.6 5 μm Alltech Alltima C18
Column: 250 × 4.6 5 μm Alltech Alltima C18
Mobile phase: MeCN:MeOH:50 mM pH 6.7 phosphate buffer 47:8:45
Column temperature: 37
Flow rate: 1
Detector: UV 263

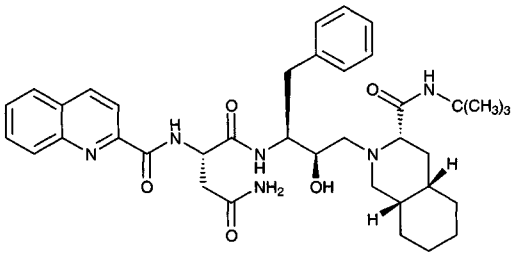
CHROMATOGRAM
Internal standard: saperconazole

OTHER SUBSTANCES
Extracted: itraconazole

KEY WORDS
serum; saperconazole is IS

REFERENCE
Christensen,K.J.; Gubbins,P.O.; Gurley,B.J.; Bowman,J.L.; Buice,R.G. Relative bioavailability of itraconazole from an extemporaneously prepared suspension and from the marketed capsules, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 261–265.

Saquinavir



Molecular formula: $C_{38}H_{50}N_6O_5$, $C_{38}H_{50}N_6O_5.CH_4O_3S$ (mesylate)
Molecular weight: 670.85
CAS Registry No.: 127779-20-8, 149845-06-7 (mesylate)
Merck Index: 8516

SAMPLE
Matrix: blood, CSF, saliva
Sample preparation: CSF, plasma. Condition a 1 mL 200 mg C2 SPE cartridge with 1 mL MeCN and 1 mL 100 mM ammonium acetate at 1 mL/min. 600 μL CSF or plasma + 600 μL 100 mm ammonium acetate solution, vortex for 10 s, centrifuge at 10500 g for 3 min. Add 1 mL of the diluted sample to the SPE cartridge using vacuum. Wash with 1 mL MeCN:100 mM ammonium acetate 30:70, suck dry using vacuum for 1 min. Elute with 400 μL MeCN:2.5 mM ammonium acetate 80:20, evaporate the eluate to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue in 150 μL mobile phase, vortex for 60 s, centrifuge at 10500 g for 3 min, inject an aliquot. Saliva. Condition a 1 mL 200 mg C2 SPE cartridge with 1 mL MeCN and 1 mL 100 mM ammonium acetate at 1 mL/min. 600 μL Saliva + 600 μL blank plasma + 1.2 mL 100 mm ammonium acetate, mix, centrifuge at 10500 g for 3 min. Add 2 mL diluted sample to the SPE cartridge using a vacuum. Wash with 1 mL MeCN:100 mM ammonium acetate 30:70, suck dry using a vacuum for 1 min. Elute with 400 μL MeCN:2.5 mM ammonium acetate 80:20, evaporate the eluate to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue in 150 μL mobile phase, vortex for 60 s, centrifuge at 10500 g for 3 min, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 Chromguard C18 (Chrompack Netherlands)
Column: 75 × 4.6 3.5 μm Zorbax SB-C18
Mobile phase: MeCN:buffer 40.5:59.5 (Buffer was water containing 25 mM sodium acetate and 25 mM hexane-1-sulfonic acid, adjusted to pH 4.0 with 37% HCl)
Flow rate: 1
Injection volume: 100
Detector: UV 239

CHROMATOGRAM
Retention time: 7.5

Limit of detection: 1.0 ng/mL
Limit of quantitation: 2.5 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Hoetelmans,R.M.W.; van Essenberg,M.; Meenhorst,P.L.; Mulder,J.W.; Beijnen,J.H. Determination of saquinavir in human plasma, saliva, and cerebrospinal fluid by ion-pair high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1997**, *698*, 235–241.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Delta-pak C4 (Waters)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mM ammonium dihydrogen phosphate and 1 mM 1-heptanesulfonic acid sodium salt, pH adjusted to 4.8 with ammonium hydroxide.)
Flow rate: 0.6
Injection volume: 35
Detector: UV 210

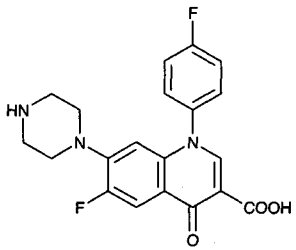
CHROMATOGRAM
Retention time: 22-27

OTHER SUBSTANCES
Simultaneous: indinavir, nelfinavir, ritonavir
Noninterfering: didanosine, lamivudine, stavudine, zalcitabine, zidovudine

REFERENCE
Iayewardene,A.L.; Zhu,F.; Aweeka,F.T.; Gambertoglio,J.G. Simple high-performance liquid chromatographic determination of the protease inhibitor indinavir in human plasma, *J.Chromatogr.B*, **1998**, *707*, 203–211.

Sarafloxacin

Molecular formula: $C_{20}H_{17}F_2N_3O_3$
Molecular weight: 385.37
CAS Registry No.: 98105-99-8, 91296-87-6 (HCl)
Merck Index: 8517



SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg Bond Elut LRC PRS SPE cartridge with 5 mL MeOH and 5 mL extracting solution 65:35. Add 25 mL extracting solution to 5 mL milk, shake for 15 s, add 4 g anhydrous sodium sulfate, shake for 15 s, centrifuge at 3000 rpm at 5° for 5 min. Remove the supernatant and repeat the extraction with 25 mL extracting solution as before except do not add any more sodium sulfate, mix mechanically, centrifuge, combine the supernatants, add 25 mL 1% acetic acid, shake for 10-15 s. Freeze for 30 min to facilitate precipitation, centrifuge at 2500 rpm at 5° for 10 min. Add 75 mL to the SPE cartridge, pass the entire sample through the cartridge, then add 2 mL MeOH, wash with 5 mL water, wash with 2 mL MeOH. Elute with 2.5 mL 25% ammonium hydroxide-MeOH. Evaporate to dryness under nitrogen at 55°, dissolve the residue in 2 mL 1% acetic acid, sonicate for 1 min, vortex for 20 s, filter (0.45 μm), inject an aliquot. (Extracting solution was 1% aqueous acetic acid:EtOH 1:99.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil
Mobile phase: MeCN:2% acetic acid 15:85
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 278 em 450, with a 418 nm cut-off filter

CHROMATOGRAM
Retention time: 5.6
Limit of detection: 1.2 ppb
Limit of quantitation: 5 ppb

OTHER SUBSTANCES
Extracted: ciprofloxacin, difloxacin, enrofloxacin

KEY WORDS
SPE

REFERENCE
Roybal,J.E.; Pfenning,A.P.; Turnipseed,S.B.; Walker,C.C.; Hurlbut,J.A. Determination of four fluoroquinolones in milk by liquid chromatography, *J.AOAC Int.*, **1997**, *80*, 982–987.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 10 mL 500 mg Bond Elut LRC PRS SPE cartridge with 2 mL MeOH and 2 mL equilibrating solution. 2 g Catfish muscle + 18 mL extracting solution, homogenize for 20 s, centrifuge at 3000 rpm for 5 min, decant the supernatant. Add another 18 mL extracting solution to the pellet and homogenize again, centrifuge at 3000 rpm for 5 min, combine the supernatants. Add 20 mL 1% glacial acetic acid, freeze for 30 min, centrifuge at 2500 rpm at 4° for 10 min. Add the extracts to the SPE cartridge, wash with 2 mL MeOH, 5 mL water, and 2 mL MeOH. Let the SPE cartridge dry for 30 s. Elute with 2 mL MeOH:30% ammonium hydroxide 80:20, dry the eluate under nitrogen at 50°. Reconstitute the residue in 500 μL mobile phase, filter (0.45 μm), inject an aliquot. (The extracting solution was EtOH:water:glacial acetic acid 98:1:1. The equilibrating solution was extracting solution:1% glacial acetic acid 35:20.)

HPLC VARIABLES
Column: 150 × 2 5 μm Inertsil Phenyl
Mobile phase: MeCN:2% formic acid 14:86
Column temperature: 40
Flow rate: 0.35
Injection volume: 50
Detector: MS, Hewlett-Packard 5989, Model 59987A electrospray, nitrogen drying gas 40 mL/min, 260°, nebulizing gas nitrogen, 80 psi, m/z 342

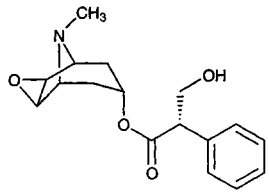
CHROMATOGRAM
Retention time: 7.33-7.75
Limit of detection: 10 ppb
Limit of quantitation: 20 ppb

OTHER SUBSTANCES
Extracted: difloxacin

KEY WORDS
catfish; muscle; SPE

REFERENCE
Turnipseed,S.B.; Walker,C.C.; Roybal,J.E.; Pfenning,A.P.; Hurlbut,J.A. Confirmation of fluoroquinolones in catfish muscle by electrospray liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1998**, *81*, 554–562.

Scopolamine



Molecular formula: $C_{17}H_{21}NO_4$
Molecular weight: 303.36
CAS Registry No.: 51-34-3, 6533-68-2 (HBr trihydrate), 114-49-8 (HBr anhydrous)
Merck Index: 8550

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL MeOH, vortex briefly, add 50 μL 1 M ammonium hydroxide, mix, add 5 mL dichloromethane, shake horizontally for 5 min, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 5 μm BDS C18 (Keystone)
Column: 50 × 3 3 μm BDS C18 (Keystone)
Mobile phase: MeCN:MeOH:10 mM ammonium acetate 62.5:37.5:15
Flow rate: 0.5
Injection volume: 20
Detector: MS, Perkin Elmer Sciex API III-Plus triple quadrupole, APCI, nebulizer 400° and 80 psi, auxiliary nitrogen 1.2 L/min, curtain gas 1.2 L/min, interface 55°, collision gas argon, electron multiplier 3000 V, declustering potential 35 V, collision energy 35 eV

CHROMATOGRAM

Retention time: 0.8
Internal standard: scopolamine

OTHER SUBSTANCES

Extracted: hyoscyamine

KEY WORDS

plasma; protect from light; scopolamine is IS

REFERENCE

Xu,A.; Havel,J.; Linderholm,K.; Hulse,J. Development and validation of an LC/MS/MS method for the determination of L-hyoscyamine in human plasma, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 33–42.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 7.39

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.9

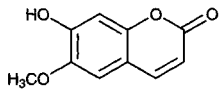
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine,

thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Scopoletin



Molecular formula: $C_{10}H_8O_4$
Molecular weight: 192.17
CAS Registry No.: 92-61-5
Merck Index: 8552

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

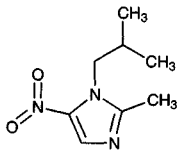
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine,

pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Secnidazole



Molecular formula: $C_7H_{11}N_3O_3$
Molecular weight: 185.18
CAS Registry No.: 3366-95-8
Merck Index: 8562

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 318.8

CHROMATOGRAM

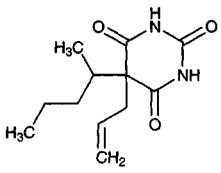
Retention time: 9.668

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Secobarbital



Molecular formula: $C_{12}H_{18}N_2O_3$
Molecular weight: 238.29
CAS Registry No.: 76-73-3, 309-43-3 (Na salt)
Merck Index: 8563
Lednicer No.: 1 269

SAMPLE

Matrix: blood
Sample preparation: 200 µL Serum + 200 µL 50 µg/mL hexobarbital in MeCN + 25 µL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 µL aliquot of the supernatant.

HPLC VARIABLES

Column: µBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM

Retention time: 25.8
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 50 µL 7 µg/mL IS in water + 1 mL buffer, vortex for 10 s, add 5 mL n-hexane:ether:n-propanol 49:49:2, shake gently for 20 min, centrifuge at 1000 g for 5 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 µL mobile phase, inject a 50-100 µL aliquot. (Buffer was 10 mM sodium acetate:10 mM acetic acid 88.5:11.5, pH 5.5.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm Partisil 5 ODS-3
Mobile phase: MeCN:buffer 28:72 (Buffer was 300 µL 1 M KH_2PO_4 and 50 µL 900 mM phosphoric acid in 1.8 L water, pH 4.4.)
Column temperature: 50
Flow rate: 2.8
Injection volume: 50-100
Detector: UV 195

CHROMATOGRAM
Retention time: 8.5
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (11.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, ethosuximide, phenytoin
Simultaneous: mephobarbital, paramethadione, phenobarbital, primidone
Noninterfering: chlorazepate, clonazepam, diazepam, thioridazine, valproic acid

KEY WORDS
serum; pharmacokinetics

REFERENCE
Levine,H.L.; Cohen,M.E.; Duffner,P.K.; Kustas,K.A.; Shen,D.D. An improved high-pressure liquid chromatographic assay for secobarbital in serum, *J.Pharm.Sci.*, **1982**, *71*, 1281–1283.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C8 SPE cartridge with 2 volumes of MeOH, 2 volumes of water, and 1 volume of 100 mM pH 5.59 Sörensen's phosphate buffer. Add 500 μL plasma to the SPE cartridge, wash with 2 volumes of 100 mM pH 5.59 Sörensen's phosphate buffer, wash with 1 volume of water, elute with 500 μL MeOH. Evaporate the eluate to dryness under vacuum, reconstitute in 50 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak C18 (Waters)
Column: 100 × 8 10 μm Radial-Pak C8 (Waters)
Mobile phase: MeOH:THF:100 mM pH 7.72 Sörensen's phosphate buffer 28:16:52
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.39
Internal standard: secobarbital

OTHER SUBSTANCES
Extracted: methohexital, pentobarbital, thiopental
Noninterfering: ketamine

KEY WORDS
plasma; dog; secobarbital is IS; SPE

REFERENCE
Avram,M.J.; Krejcie,T.C. Determination of sodium pentobarbital and either sodium methohexital or sodium thiopental in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1987**, *414*, 484–491.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)

Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 7.31
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, theophylline, thiopental
Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid
Interfering: methyl salicylate

KEY WORDS
serum

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 13.80
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with an equal volume of MeCN, centrifuge at 10000 g, dilute supernatant with an equal volume of water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 110 × 4.7 5 μm PartiSphere C18 (Whatman)
Mobile phase: MeCN:15 mM pH 7.0 phosphate buffer 30:70
Flow rate: 0.8
Injection volume: 50
Detector: UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted coil of PTFE tubing irradiated by an 8 W low-pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 8.7

OTHER SUBSTANCES
Extracted: aprobarbital, butethal, pentobarbital

KEY WORDS
plasma; post-column reaction; post-column photochemical derivatization

REFERENCE
Wolf,C.; Schmid,R.W. Enhanced UV-detection of barbiturates in HPLC analysis by on-line photochemical reaction, *J.Liq.Chromatogr.*, **1990**, *13*, 2207–2216.

SAMPLE
Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 12.82
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 17.42

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Dissolve injection in mobile phase to give a secobarbital sodium concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 75 × 4.6 5 μm Chromegabond C18 or 150 × 4.6 7 μm Zorbax ODS

Mobile phase: MeOH:buffer:polyethylene glycol 300 60:40:0.4 (Buffer was 4.1 g anhydrous sodium acetate and 15 mL acetic acid in 1 L water.)

Flow rate: 1

Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
rugged; injections

REFERENCE
Reif,V.D.; Kaufmann,K.L.; DeAngelis,N.J.; Frankhouser,M.C. Liquid chromatographic assays for barbiturate injections, *J.Pharm.Sci.*, **1986**, *75*, 714–716.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 20-200 μg/mL solution in acetone with 50 μL of a 0.4-1.6 mg/mL solution of 2-bromo-2'-acetonaphthone in acetone, add 5-10 mg cesium carbonate, heat at 30° for 30 min, add 50 μL glacial acetic acid, mix, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 2
Detector: UV 249

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: amobarbital, butobarbital, heptobarbital, hexobarbital, mephobarbital, pentobarbital, phenobarbital
Interfering: barbital

KEY WORDS
derivatization

REFERENCE
Hulshoff,A.; Roseboom,H.; Renema,J. Improved detectability of barbiturates in high-performance liquid chromatography by pre-column labelling and ultraviolet detection, *J.Chromatogr.*, **1979**, *186*, 535–541.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate a solution in water, MeOH, or diethyl ether to dryness, add a 3-fold molar excess of triethylamine, add 0.5-3 mL MeCN, add a 3-fold molar excess of N-chloromethyl-4-nitrophthalimide, heat at 60° for 1 h, inject an aliquot. (Preparation of N-chloromethyl-4-nitrophthalimide is as follows. Suspend 130 g 4-nitrophthalimide in 80 mL 40% formaldehyde solution, add 200 mL water, reflux for 4 h, filter while hot, N-(hydroxymethyl-4-nitrophthalimide crystallizes on cooling (cf. J. Am. Chem. Soc. 1922, 44, 817). Mix a suspension of 2.26 g N-hydroxymethyl-4-nitrophthalimide in 10-15 mL ether with a suspension of 2.1 g phosphorus pentachloride in 10-15 mL ether, after 10 min heat on a water bath, cool in an ice-salt mixture, add ice-water dropwise with shaking, filter to obtain N-chloromethyl-4-nitrophthalimide, dry under vacuum (cf. Chem. Ber. 1959, 9, 1258).)

HPLC VARIABLES
Column: 7 μm LiChrosorb RP8

Mobile phase: MeCN:water 60:40
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.6
Limit of detection: 4 ng

OTHER SUBSTANCES
Extracted: amobarbital
Simultaneous: cyclobarbital, methylphenobarbital, phenobarbital

KEY WORDS
derivatization

REFERENCE
Lindner,W.; Santi,W. N-chloromethylphthalimides as derivatization reagents for high-performance liquid chromatography, *J.Chromatogr.*, **1979**, *176*, 55–64.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:5 mM sodium carbonate 9:81. B was MeCN:20 mM sodium carbonate 20:80. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: allobarbital, amobarbital, barbital, barbituric acid, butabarbital, mephobarbital, methabarbital, methohexital, phenobarbital, phenytoin, thiamylal

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mm KH_2PO_4 + 5 mM 1-decanesulfonic acid 30:70, adjusted to pH 3.2 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 13.6
Internal standard: methyl paraben (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, butalbital, aprobarbital, mephobarbital, pentobarbital, phenobarbital, talbutal, vinbarbital

KEY WORDS
stability-indicating

REFERENCE
Ibrahim,F.B. Simultaneous determination and separation of several barbiturates and analgesic products by ion-pair high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2835–2851.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 2.04

OTHER SUBSTANCES
Simultaneous: aprobarbital, pentobarbital, amobarbital, butabarbital, butalbital, thiopental, phenobarbital

REFERENCE
Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, imino-

stilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.19 (A), 5.91 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, met-

ronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.17

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 1.0
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone
Interfering: caffeine, cotinine, benzoylecgonine, oxazepam, phenobarbital, nordiazepam

KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine
Sample preparation: 2 mL Urine +1 mL 500 mM pH 5.5 phosphate buffer, add to an Extrelut 3 SPE cartridge, let stand for 10 min, elute with 15 mL dichloromethane:isopropanol 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm Lichrospher 100 RP8
Column: 250 × 4 5 μm Lichrospher 100 RP8
Mobile phase: Gradient. MeCN:10 mM pH 4.4 phosphate buffer from 30:70 to 40:60 over 8 min, maintain at 40:60 for 6 min, to 30:70 over 1 min
Flow rate: 1
Injection volume: 20
Detector: UV 212

CHROMATOGRAM

Retention time: 13.0
Limit of detection: 150 ng/mL

OTHER SUBSTANCES

Extracted: barbital, allobarbital, butabarbital, phenobarbital, pentobarbital
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nic-

otine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, triflupromazine, trimethoprim, trimipramine

KEY WORDS
SPE

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

Secretin

Molecular formula: $C_{130}H_{220}N_{44}O_{41}$
Molecular weight: 3055.45
CAS Registry No.: 1393-25-5
Merck Index: 8564

1 9
His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-
10 18
Leu-Ser-Arg-Leu-Arg-Asp-Ser-Ala-Asp-
19 27
Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in 1% trifluoroacetic acid adjusted to pH 2.5 with triethylamine, filter (0.2 μm), inject a 80 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Whatman)
Column: 300 mm long MCH-10 C18 (Varian)
Mobile phase: Gradient. A was 1% trifluoroacetic acid adjusted to pH 2.5 with triethylamine. B was isopropanol:trifluoroacetic acid 99:1 containing the same amount of triethylamine as A. A:B 80:20 for 5 min, to 65:35 over 50 min, to 40:60 over 10 min, maintain at 40:60 for 10 min. At the end of each run cycle repeatedly from 60:40 to 40:60 for 15 min.
Flow rate: 0.5
Injection volume: 80
Detector: RIA of fractions

CHROMATOGRAM
Retention time: 60

OTHER SUBSTANCES
Simultaneous: motilin, sincalide

REFERENCE
Chang,T.-M.; Erway,B.; Chey,W.Y. Rapid, small-scale preparation of gastrointestinal hormones by high-performance liquid chromatography on a C18 column, *J.Chromatogr.*, **1985**, *326*, 121–127.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in water, inject a 2 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:0.01% HCl 25:65
Flow rate: 1
Injection volume: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Tsuda,T.; Uchiyama,M.; Sato,T.; Yoshino,H.; Tsuchiya,Y.; Ishikawa,S.; Ohmae,M.; Watanabe,S.; Miyake,Y. Identification of secretin diastereoisomers produced during synthesis, *J.Pharm.Sci.*, **1989**, *78*, 91–94.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL Secretin in pH 4 or 7 Buffer (μ = 0.5) + 300 μL MeCN:1% perchloric acid 40:60, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 5 mM pH 3 phosphate buffer containing 200 mM sodium perchlorate.)
Flow rate: 1
Injection volume: 10
Detector: UV 210

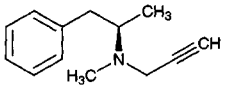
CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
buffer

REFERENCE
Tsuda,T.; Uchiyama,M.; Sato,T.; Yoshino,H.; Tsuchiya,Y.; Ishikawa,S.; Ohmae,M.; Watanabe,S.; Miyake,Y. Degradation peptides of secretin after storage in acid and neutral aqueous solutions, *J.Pharm.Sci.*, **1990**, *79*, 53–56.

Selegiline



Molecular formula: $C_{13}H_{17}N$
Molecular weight: 187.28
CAS Registry No.: 2323-36-6, 14611-51-9
Merck Index: 8569

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM
Retention time: 10.712

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM $(NH_4)_3PO_4$ 20:80 adjusted to pH 3.1 with concentrated phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 1 (relative retention time)
Internal standard: methamphetamine (relative retention time = 0.71)

REFERENCE
Vargay,Z.; Horváth,G.; Korponay,K.; Kovács,G.; Kálmánne Máthé,I.; Bánki,A. Attekintes a selegilin hatóanyag analitikájáról [Survey of the analysis of selegiline], *Acta Pharm.Hung.*, **1992**, *62*, 212–217.

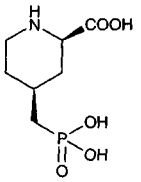
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 3 μm Microsorb RP-18
Mobile phase: MeCN:buffer 20:80 (Buffer was 100 mM $(NH_4)H_2PO_4$ and 0.08% triethylamine adjusted to pH 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 205

OTHER SUBSTANCES
Simultaneous: degradation products, methamphetamine

REFERENCE
Chafetz,L.; Desai,M.P.; Sukonik,L. Trace decomposition of selegiline. Use of worst-case kinetics for a stable drug, *J.Pharm.Sci.*, **1994**, *83*, 1250–1252.

Selfotel



Molecular formula: $C_7H_{14}NO_5P$
Molecular weight: 223.17
CAS Registry No.: 110347-85-8

SAMPLE

Matrix: urine

Sample preparation: 50 μL Urine + 25 μL 100 μg/mL IS in water + 1 mL buffer, vortex for 5 s, add 100 μL reagent, vortex for 10 s, let stand for 1.5 min, add 200 μL 100 mg/mL iodoacetamide in MeCN, vortex for 15 s, let stand for 1.5 min, add 500 μL 2 mg/mL 9-fluorenylmethyl chloroformate in acetone, vortex for 20 s, let stand for 2 min, add 5 mL ether, vortex for 1 min, repeat the ether wash. Remove traces of organic solvent from the aqueous layer with a stream of nitrogen, vortex the aqueous layer for 2 s. Remove a 50 μL aliquot and add it to 500 μL mobile phase, inject a 50 μL aliquot onto column A and elute to waste with mobile phase A, divert the fraction containing selfotel and the IS onto column B, after 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Prepare buffer by dissolving 15.5 g boric acid in 500 mL water, adjust pH to 9.5 with NaOH. Prepare reagent by adding 32 μL 3-mercaptopropionic acid to 1 mL 50 mg/mL o-phthalaldehyde in MeCN. Primary amino acids are removed by derivatization with o-phthalaldehyde/3-mercaptopropionic acid.)

HPLC VARIABLES

Column: A 75 × 4.6 5 μm Inertsil ODS-2; B 250 × 4.6 10 μm Chiralcel OD-R

Mobile phase: A MeCN:100 mM pH 2.50 phosphate buffer 35:65; B MeCN:100 mM pH 2.00 phosphate buffer 35:65

Column temperature: 30

Flow rate: A 0.5 for 16 min, to 2.0 over 0.3 min, maintain at 2.0 for 18.4 min, return to 0.5 over 0.3 min; B 0.5

Injection volume: 50

Detector: F ex 262 em 314

CHROMATOGRAM

Retention time: 19.2, 22.0 (enantiomers)

Internal standard: cis-4-(phosphonoethyl)-2-piperidinecarboxylic acid (CGS 20005) (36 (first eluting peak))

Limit of quantitation: 250 ng/mL

KEY WORDS

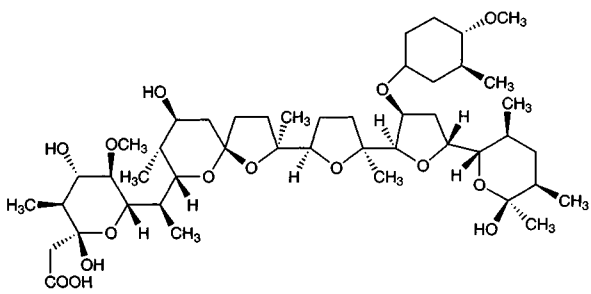
derivatization; chiral; column-switching

REFERENCE

Knoche,B.; Milosavljev,S.; Gropper,S.; Brunner,L.A.; Powell,M.L. Enantioselective determination of selfotel in human urine by high-performance liquid chromatography on a chiral stationary phase after derivatization with 9-fluorenylmethyl chloroformate, *J.Chromatogr.B*, **1997**, *695*, 355–363.

Semduramicin

Molecular formula: $C_{45}H_{76}O_{16}$
Molecular weight: 873.09
CAS Registry No.: 113378-31-7, 119068-77-8 (sodium salt)
Merck Index: 8587



SAMPLE

Matrix: tissue

Sample preparation: Condition a 200 mg BondElut LRC C8 SPE cartridge with 5 mL MeCN, 5 mL MeOH, add 5 mL water, do not allow to dry. Condition a 500 mg BondElut LRC silica SPE cartridge with 5 mL chloroform and 5 mL isooctane:dichloromethane 50:50, do not allow to dry. Vortex 1.25 g homogenized liver with 7.5 mL MeOH:water:ammonium hydroxide 80:20:1 for 3 min, heat at 55° for 1 h, centrifuge at 4000 rpm for 5 min, decant the supernatant, rinse the tube with 1-2 mL MeOH. Combine the rinse and the supernatant and evaporate them to 2-3 mL under a stream of nitrogen at 55°, add 5 mL water, vortex, sonicate for 5 min, add to the C8 SPE cartridge, rinse the tube with 1-2 mL water, add the rinse to the C8 SPE cartridge, wash with 3 mL water, wash with 1 mL MeOH:water 25:75, elute with 5 mL ethyl acetate, evaporate to dryness under a stream of nitrogen at 55°, reconstitute with 6 mL isooctane:dichloromethane 50:50, vortex, sonicate for 5 min, add to the silica SPE cartridge, rinse the tube with 1.5 mL isooctane:dichloromethane 50:50, add the rinse to the SPE cartridge, wash with 2.5 mL isooctane:dichloromethane 50:50, wash with 1 mL ethyl acetate, elute with 5 mL dichloromethane:MeOH 90:10, evaporate to dryness under a stream of nitrogen at 55°, reconstitute with 150 μL isooctane:ethyl acetate 60:40, vortex, sonicate for 2 min, inject a 75 μL aliquot.

HPLC VARIABLES

Guard column: 20 mm long 40 μm LC-Si (Supelco)
Column: 250 × 4.6 Zorbax silica
Mobile phase: Isooctane:ethyl acetate:glacial acetic acid:triethylamine:MeOH 35:65:0.4:0.2:0.1 (At the end of each day flush column with isooctane:ethyl acetate 40:60 for at least 3 h.)
Flow rate: 0.6
Injection volume: 75
Detector: UV 522 following post-column reaction. The column effluent mixed with the reagent pumped at 0.3 mL/min and the mixture flowed through a 15 m × 0.25 mm ID stainless steel coil at 95 ± 1° to the detector. At the end of each day flush the pump with MeOH for at least 3 h. (Prepare reagent by cautiously adding 20 mL concentrated sulfuric acid to 500 mL EtOH, cool to room temperature, add 30 g vanillin dissolved in 500 mL EtOH, mix, protect from light, prepare every other day.)

CHROMATOGRAM

Retention time: 11.3
Limit of detection: 25 ng/g

OTHER SUBSTANCES

Simultaneous: maduramicin, monensin, narasin, salinomycin

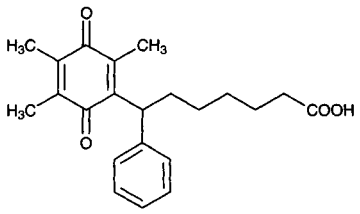
KEY WORDS

post-column reaction; normal phase; SPE; chicken; liver

REFERENCE

Ericson,J.F.; Calcagni,A.; Lynch,M.J. Determination of senduramicin sodium in poultry liver by liquid chromatography with vanillin postcolumn derivatization, *J.AOAC Int.*, **1994**, *77*, 577–582.

Seratrodast



Molecular formula: $C_{22}H_{26}O_4$
Molecular weight: 354.45
CAS Registry No.: 112665-43-7
Merck Index: 8603

SAMPLE

Matrix: blood
Sample preparation: Add IS to plasma, acidify with 300 mM HCl. Extract the samples with 5 mL hexane:ethyl acetate 20:80, evaporate the organic layer to dryness, reconstitute with 150 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm Adsorbosphere CN
Column: 250 × 4.6 5 μm Phenomenex PhenoSphere C8
Mobile phase: MeCN:MeOH:isopropanol:1M pH 4.0 sodium perchlorate 35:20:5:40
Flow rate: 1.0
Injection volume: 100
Detector: UV 266

CHROMATOGRAM

Internal standard: A-68500
Limit of quantitation: 3.3 ng/mL

KEY WORDS

plasma

REFERENCE

el-Shourbagy,T.; Hsu-Beischer,R.; Sapochak,L.; Chu,S. An HPLC method for the simultaneous determination of CEP-2563 (KT-8391) and its active metabolites CEP-2547, CEP-751, and CEP-701, in human plasma using fluorometric detection (Abstract 3354), *Pharm.Res.*, **1997**, *14*, S582.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 150 μL 100 mM HCl to 500 μL serum, add 6 mL 167 ng/mL idebenone in ethyl acetate, extract, centrifuge, add 200 μL 5% propylene glycol in ethyl acetate to the organic layer, evaporate to dryness under a stream of nitrogen at 40°, add 2 mL iron(III) chloride solution to the residue, vortex for 10 s. Add 4 mL ethyl acetate, extract, add 200 μL 5% propylene glycol solution to the separated organic layer, evaporate to dryness under a stream of nitrogen at 40°, dissolve the residue in 300 μL MeCN:50 mM KH_2PO_4 55:45, inject a 100 μL aliquot. Urine. Add 750 μL enzyme solution (667 U/mL β-glucuronidase (type B-3, H-1, and IX) and 33.3 U/mL sulfatase (type VIII) in 200 mM pH 5.0 phosphate buffer) to 250 μL urine, incubate the mixture at 37° for 1 h. Add 6 mL 167 ng/mL idebenone in ethyl acetate solution to the hydrolyzed urine or to 500 μL urine, extract, centrifuge, add 200 μL 5% propylene glycol in ethyl acetate to the organic layer, evaporate to dryness under a stream of nitrogen at 40°, add 2 mL iron(III) chloride solution to the residue, vortex for 10 s. Add 4 mL ethyl acetate, extract, add 200 μL 5% propylene glycol in ethyl acetate to the separated organic layer, evaporate to dryness under a stream of nitrogen at 40°, dissolve the residue in 300 μL MeCN:50 mM pH 3.0 phosphate buffer 55:45, inject a 100 μL aliquot. (The compounds are oxidized by the iron(III) chloride.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm YMC Pack ODS, A-302
Mobile phase: Gradient. A was 50 mM KH_2PO_4 (serum) or 50 mM pH 3.0 phosphate buffer (urine). B was MeCN:50 mM KH_2PO_4 60:40 (serum) or MeCN:50 mM pH 3.0 phosphate buffer 60:40 (urine). A:B from 50:50 to 0:100 in 50 min, maintain at 0:100 for 5 min, from 0:100 to 50:50 for 5 min, maintain at 50:50 for 15 min
Column temperature: 25

Flow rate: 1
Injection volume: 100
Detector: UV 266

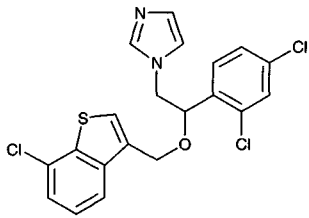
CHROMATOGRAM
Retention time: 44.5
Internal standard: idebenone (40)
Limit of detection: 5 ng/mL (serum, urine), 10 ng/mL (hydrolyzed urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics; serum; derivatization

REFERENCE
Ohta,R.; Amano,T.; Yamashita,K.; Motohashi,M. High-performance liquid chromatographic determination of seratrodast and its metabolites in human serum and urine, *J.Chromatogr.B*, **1997**, *704*, 325–331.

Sertaconazole



Molecular formula: $C_{20}H_{15}Cl_3N_2OS$
Molecular weight: 437.78
CAS Registry No.: 99592-32-2
Merck Index: 8610

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 96 μg/mL solution in MeCN, inject a 25 μL aliquot. Cream. Disperse 2 g cream in 15 mL MeCN:MeOH 80:20 using a spatula, sonicate for 10 min, make up to 50 mL with MeCN, centrifuge at 4500 rpm for 10 min. Dilute 1 mL of the supernatant to 10 mL with MeCN, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Spherisorb CN
Mobile phase: MeCN:10 mM NaH_2PO_4 37:63
Column temperature: 35
Flow rate: 1.6
Injection volume: 25
Detector: UV 260

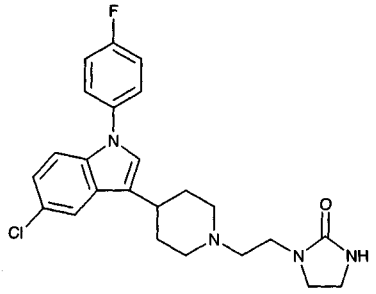
CHROMATOGRAM
Retention time: 19.3

OTHER SUBSTANCES
Simultaneous: impurities, degradation products

KEY WORDS
cream

REFERENCE
Albet,C.; Fernandez,J.M.; Rozman,E.; Perez,J.A.; Sacristan,A.; Ortiz,J.A. Determination of sertaconazole nitrate, a new imidazole antifungal, by high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 205–211.

Sertindole



Molecular formula: $C_{24}H_{26}ClFN_4O$
Molecular weight: 440.95
CAS Registry No.: 106516-24-9
Merck Index: 8611

SAMPLE

Matrix: blood

Sample preparation: Condition a 200 mg Bond Elut C8 SPE cartridge with two 2 mL portions of MeCN, three 3 mL portions of MeOH, and two 2 mL portions of water. Briefly vortex 1 mL (human), 500 μL (dog), 200 μL (rat), or 100 μL (mouse) plasma with 50 μL 100 ng/mL IS in EtOH and 10 mM pH 8.5 dibasic potassium phosphate as required for sample transfer. Add to the SPE cartridge, wash with three 2 mL portions of water, and with three 1 mL portions of MeCN, dry with vacuum for 1-2 min. Elute with two 1 mL portions of MeOH:glacial acetic acid 98:2, evaporate the eluate to dryness under a gentle stream of air or nitrogen at 25-35°. Reconstitute the residue with 50 μL mobile phase, centrifuge at 3000 rpm for 5 min, maintain extract at 5° or less until injection, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: guard cartridge
Column: 150 × 2.1 5 μm YMC basic (YMC Inc., Wilmington, NC)
Mobile phase: MeCN:100 mM ammonium acetate 42:58 adjusted to pH 6.8 with glacial acetic acid
Flow rate: 0.4
Injection volume: 20
Detector: MS, Sciex API III, positive ion mode at 450°, nebulizing gas nitrogen 80 psi, auxiliary gas nitrogen at 2-3 mL/min, curtain gas nitrogen at 1.8 mL/min, m/z 441-113

CHROMATOGRAM

Retention time: 3.9
Internal standard: Lu-26-009
Limit of quantitation: 100 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

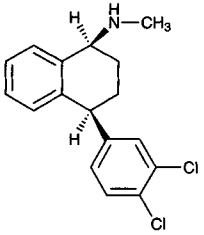
KEY WORDS

SPE; plasma; human; rat; dog; mouse; pharmacokinetics

REFERENCE

Menacherry,S.D.; Stamm,G.E.; Chu,S.-Y. A sensitive and specific method for assay of sertindole and its metabolites in human, rat, dog, and mouse plasma using HPLC with tandem mass spectrometric detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2241–2257.

Sertraline



Molecular formula: $C_{17}H_{17}Cl_2N$
Molecular weight: 306.23
CAS Registry No.: 79617-96-2, 79559-97-0 (HCl)
Merck Index: 8612
Lednicer No.: 4 57

SAMPLE

Matrix: blood, tissue, vitreous humor
Sample preparation: Blood, vitreous humor. Mix 1 mL sample with 500 ng IS, add 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 200 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer. Tissue. Mix 500 μL liver homogenate with 5.0 μg IS, add 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 400 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 3.9 5 μm NovaPak-Phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 55:45, adjusted to pH 3.0
Flow rate: 1.5
Injection volume: 30
Detector: UV 214

CHROMATOGRAM

Internal standard: pentazocine
Limit of quantitation: 100 ng/mL (blood), 2.5 μm/g (liver)

OTHER SUBSTANCES

Extracted: pimozide

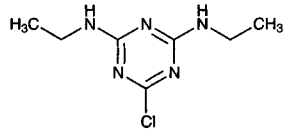
KEY WORDS

liver

REFERENCE

McIntyre,I.M.; King,C.V.; Staikos,V.; Gall,J.; Drummer,O.H. A fatality involving moclobemide, sertraline, and pimozide, *J.Forensic Sci.*, **1997**, *42*, 951–953.

Simazine



Molecular formula: $C_7H_{12}ClN_5$
Molecular weight: 201.66
CAS Registry No.: 122-34-9
Merck Index: 8681

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

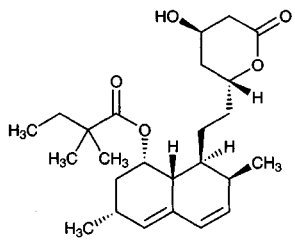
CHROMATOGRAM
Retention time: 15.752

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Simvastatin

Molecular formula: $C_{25}H_{38}O_5$
Molecular weight: 418.57
CAS Registry No.: 79902-63-9
Merck Index: 8686



SAMPLE
Matrix: blood
Sample preparation: Condition a 2.8 mL 500 mg Bond Elut C8 SPE cartridge with 2 mL MeOH and 2.5 mL water. Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with 1.5 mL MeCN and 2 mL water. 1 mL Plasma + 2.5 ng IS, mix, add to the C8 SPE cartridge, wash with 2 mL MeCN:water 10:90, wash with 1 mL MeOH:water 30:70, wash with 2 mL MeOH:water 60:40 (the ring-opened active metabolite elutes in this fraction), elute with 2 mL MeCN, add 100 μL 20 mM potassium carbonate to the eluate, evaporate to dryness under reduced pressure at 40° (this hydrolyses the lactone), dry under vacuum for more than 30 min, reconstitute with 100 μL 10 mM 1-(bromoacetyl)pyrene in DMF, add 100 μL 10 mM 18-crown-6 in DMF, mix, let stand at room temperature for 30 min, add 2 mL MeCN:triethylamine 90:10, add to a 10 mL 100 mg Bond Elut LRC PBA SPE cartridge, wash with 4 mL MeOH, wash with 2 mL MeCN, elute with 2 mL MeCN:propylene glycol 60:40, dilute the eluate with 1 mL water, add to the C18 SPE cartridge, wash with 2 mL MeCN:water 70:30, elute with 3 mL MeCN. Evaporate the eluate to dryness, reconstitute the residue in 300 μL MeCN:water 70:30, inject a 150 μL aliquot onto column A and elute to waste with mobile phase A, after 12.5 min elute column A onto column B with mobile phase A, after another 5 min remove column A from the circuit and elute column B with mobile phase B, monitor the effluent from column B. Flush column A with MeOH then re-equilibrate with mobile phase A for 6 min. (The procedure can also be modified to determine the active metabolite.)

HPLC VARIABLES
Column: A 150 × 4.6 5 μm Bondesil CH (Varian); B 150 × 4.6 5 μm Capcell Pak C18 UG 120 (Shiseido)

Mobile phase: A MeOH:water 80:20; B MeCN:water 80:20
Column temperature: 40
Flow rate: 1
Injection volume: 150
Detector: F ex 360 em 430

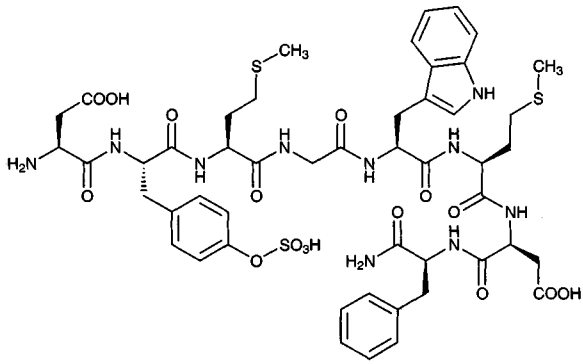
CHROMATOGRAM
Retention time: 27.5
Internal standard: 2-ethyl-2-methylbutanoate ester analog of simvastatin (31)
Limit of detection: 20 pg/mL

KEY WORDS
derivatization; plasma; column-switching; SPE; heart-cut; pharmacokinetics

REFERENCE
Ochiai,H.; Uchiyama,N.; Imagaki,K.; Hata,S.; Kamei,T. Determination of simvastatin and its active metabolite in human plasma by column-switching high-performance liquid chromatography with fluorescence detection after derivatization with 1-bromoacetylpyrene, *J.Chromatogr.B*, **1997**, *694*, 211–217.

Sincalide

Molecular formula: $C_{49}H_{62}N_{10}O_{16}S_3$
Molecular weight: 1143.29
CAS Registry No.: 25126-32-3
Merck Index: 8689



SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeCN and 10 mL 0.1% acetic acid. Add 3 mL plasma to the SPE cartridge, wash with 5 mL 0.1% acetic acid, elute with 6 mL MeCN:0.1% acetic acid 50:50, lyophilize the eluate. Reconstitute with 400 μL water:0.1% trifluoroacetic acid 50:50, filter (Millex-HV, Millipore), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK ODS-120 T
Mobile phase: MeCN:0.1% trifluoroacetic acid 34:66
Flow rate: 1
Detector: UV 214 or RIA

CHROMATOGRAM
Retention time: 6.5

KEY WORDS
plasma; SPE; dog

REFERENCE
Lindén,A.; Uvnäs-Moberg,K. Plasma levels of cholecystokinin (CCK-8 and CCK-33-39) in response to feeding and during pregnancy in dogs, *Scand.J.Gastroenterol.*, **1987**, *22*, 859–864.

SAMPLE
Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeCN and 10 mL 0.1% acetic acid. Add 2 mL plasma to the SPE cartridge, wash with 5 mL 0.1% acetic acid, elute with 6 mL MeCN:0.1% acetic acid 50:50, lyophilize the eluate. Reconstitute with 200 μL 1% pH 8.0 ammonium bicarbonate, incubate with 5 μg protease (from Staphylococcus aureus V.8, Miles Scientific) at 37° for 6 h, lyophilize. Reconstitute with 500 μL water:0.1% trifluoroacetic acid 50:50, filter (Millex-HV, Millipore), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK ODS-120 T (LKB)
Mobile phase: MeCN:0.1% trifluoroacetic acid containing 155 mM NaCl 32:68
Flow rate: 1
Detector: UV 215 or RIA

CHROMATOGRAM
Retention time: 9.5

KEY WORDS
rat; plasma; SPE

REFERENCE
Lindén,A.; Carlquist,M.; Hansen,S.; Uvnäs-Moberg,K. Plasma concentrations of cholecystokinin, CCK-8, and CCK-33, 39 in rats, determined by a method based on enzyme digestion of gastrin before HPLC and RIA detection of CCK, *Gut*, **1989**, *30*, 213–222.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in 1% trifluoroacetic acid adjusted to pH 2.5 with triethylamine, filter (0.2 μm), inject a 80 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Whatman)
Column: 300 mm long MCH-10 C18 (Varian)
Mobile phase: Gradient. A was 1% trifluoroacetic acid adjusted to pH 2.5 with triethylamine. B was isopropanol:trifluoroacetic acid 99:1 containing the same amount of triethylamine as A. A:B 80:20 for 5 min, to 65:35 over 50 min, to 40:60 over 10 min, maintain at 40:60 for 10 min. At the end of each run cycle repeatedly from 60:40 to 40:60 for 15 min.
Flow rate: 0.5
Injection volume: 80
Detector: RIA of fractions

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Simultaneous: motilin, secretin

REFERENCE
Chang,T.-M.; Erway,B.; Chey,W.Y. Rapid, small-scale preparation of gastrointestinal hormones by high-performance liquid chromatography on a C18 column, *J.Chromatogr.*, **1985**, *326*, 121–127.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) frozen brain tissue with 5 volumes of ice-cold MeOH:water 90:10, centrifuge at 10000 g for 5 min, lyophilize the supernatant, reconstitute with 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 7 μm LiChrosorb RP-18
Mobile phase: Isopropanol:150 mM pH 5.5 phosphate buffer 11.5:88.5
Column temperature: 45
Flow rate: 0.6

Injection volume: 20
Detector: E, BAS LC-4, TL-5 glassy carbon electrode +1.0 V, Ag/AgCl reference electrode

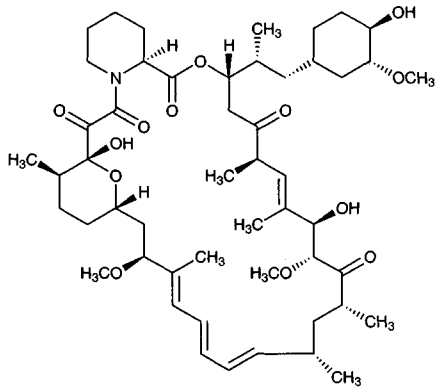
CHROMATOGRAM
Retention time: 5
Limit of quantitation: 0.2 ng

KEY WORDS
rat; brain

REFERENCE
Sauter,A.; Frick,W. Determination of cholecystokinin tetrapeptide and cholecystokinin octapeptide sulfate in different rat brain regions by high-pressure liquid chromatography with electrochemical detection, *Anal.Biochem.*, **1983**, *133*, 307–313.

Sirolimus

Molecular formula: $C_{51}H_{79}NO_{13}$
Molecular weight: 914.19
CAS Registry No.: 53123-88-9
Merck Index: 8288



SAMPLE
Matrix: blood, tissue
Sample preparation: Vortex 0.05-1 mL whole blood or hepatic microsomes with 1 μg IS and 1 mL 100 mM sodium carbonate for 10 s. Add 10 mL MTBE, shake horizontally for 30 min. Centrifuge at 1500 g for 10 min at 4°, remove the organic layer, evaporate to dryness under a stream of nitrogen. Reconstitute the residue in 200 μL mobile phase. Centrifuge at 2500 g at 4°. Inject a 150 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 mm 37-53 μm C18 pellicular (Upchurch)
Column: 250 × 4.6 5 μm Supelco LC-318 C18
Mobile phase: MeOH:water 70:30
Column temperature: 45
Flow rate: 1.0
Injection volume: 150
Detector: UV 278

CHROMATOGRAM
Retention time: 22, 32 (isomers)
Internal standard: N-undecyl-o-toluamide (Prepare by dispersing N-undecylamine in cold NaOH and adding an equimolar amount of o-toluoyl chloride, shake vigorously. Remove the product by filtration, wash, air dry, recrystallize from EtOH/water.) (27.5)
Limit of detection: 1 ng
Limit of quantitation: 2.5 ng

OTHER SUBSTANCES
Simultaneous: beclomethasone, corticosterone, cyclosporine A and G, erythromycin, ethinyl estradiol, hydrocortisone, ketoconazole, lorazepam, methylprednisolone, norethindrone, prednisolone, prednisone, propranolol, rifampicin, tacrolimus